

Oral Administration of D-aspartate, but not of L-aspartate, Reduces Food Intake in Chicks

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In the present study, we determined the effects of oral administration of L- and D-aspartate (L-Asp and D-Asp) on food intake over a period of 2 h after the administration, as well as its effects on the concentration of L- and D-Asp in the brain and plasma. Chicks were orally administered different levels (0, 3.75, 7.5 and 15 mmol/kg body weight) of L-Asp (Experiment 1) and D-Asp (Experiment 2). Administration of several doses of L-Asp linearly increased the concentration of L-Asp, but not of D-Asp, in plasma. Oral L-Asp somewhat modified the levels of L- and D-Asp levels in the telencephalon, but not in the diencephalon. However, food intake was not significantly changed with doses of L-Asp. On the other hand, D-Asp strongly and dose-dependently inhibited food intake over a period of 2 h after the administration. Oral D-Asp clearly increased D-Asp levels in the plasma and diencephalon, but no significant changes in L-Asp were detected. Brain monoamine contents were only minimally influenced by L- or D-Asp administration. We conclude that D-Asp may act as an anorexigenic factor in the diencephalon.

Key words: brain, D-Aspartate, food intake, L-Aspartate, neonatal chick, plasma

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Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and mediates its action via the activation of several subtypes of excitatory amino acid (EAA) receptors including the *N*-methyl-D-aspartate glutamate (NMDA) receptor (Kubrusly *et al.*, 1998). Past studies have determined the brain neurochemical systems that regulate food intake, and have identified a number of neurotransmitters that appear to contribute to this behavior. For example, L-aspartate (L-Asp) and its enantiomer D-aspartate (D-Asp) are EAA members, and have been well documented as regulators of the endocrine system (Estienne *et al.*, 1997; D'Aniello *et al.*, 2000a,b); as such, they may affect the growth performance of domestic animals.

D-Asp, synthesized from L-Asp by aspartate racemase,

can directly stimulate the NMDA receptor (D'Aniello *et al.*, 2000a; Woloskor *et al.*, 2000). Additionally, numerous studies involving pharmacological treatments have investigated the role of glutamate in the regulation of appetitive behavior in mammals. Intracerebroventricular (i.c.v.) administration or local administration of glutamate (Reddy *et al.*, 1986; Ritter and Stone, 1987; Wandji *et al.*, 1989) or the glutamate agonist, NMDA, induced hyperphagia, but these effects were not elicited by the administration of kainate or alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) in chicks (Bungo *et al.*, 2011). These orexigenic effects of glutamate and NMDA appear to be mediated not only by NMDA receptors but also by other EAA receptor subtypes (Da Silva *et al.*, 2003). Although these reports suggest that L-Asp and D-Asp might also have an orexigenic effect in chicks through the activation of the NMDA receptor in the brain, Bungo *et al.* (2002) demonstrated that i.c.v. injection of L-Asp as a glutamate receptor agonist did not alter eating behaviors in chicks. In addition, numerous studies have also investigated the role of D-Asp in the regulation of food intake in mammals. For instance,

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Koyuncuoğlu *et al.* (1982) reported that orally administered D-Asp caused significant decreases in body weight and the weight of the liver, as well as in daily food intake in rats. As with mammals, it was reported that D-Asp in the diet reduced food intake in chicks (Maruyama *et al.*, 1972). In fact, L- and D-Asp have been shown to exist in various brain regions of the chicken (Neidle and Dunlop, 1990) and the pigeon (Kera *et al.*, 1996). However, little is known about the mechanisms underlying the effects of either L- or D-Asp on food intake in chicks. The purpose of the present study was to examine the effects of the oral administration of different doses of L- and D-Asp on food intake in chicks. We explored this issue further by also investigating the effects of these treatments on the concentration of L- and D-Asp in plasma and the brain. The effects of L-Asp and D-Asp on levels of brain monoamines (dopamine [DA], serotonin [5-HT] and their metabolites) were also investigated, since these monoamines have been recognized as regulators of behavior and/or of stress responses in chicks (Zhang *et al.*, 2003; Saito *et al.*, 2004; Hamasu *et al.*, 2009).

Materials and Methods

Animals and Drugs

One-day-old layer chicks were purchased from a local hatchery (Murata hatchery, Fukuoka, Japan) and housed in a wire-meshed cage (50 × 35 × 33 cm) in a group (20–25 birds) at a constant temperature of 30 ± 1°C and with continuous light until the day of the experiment. Chicks were all of the same age and were housed without an adult. Food (AX, Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water were available *ad libitum*. One day before the experiment, chicks (4 days old) were reared individually and assigned to treatment groups on the basis of their body weight in order to produce uniform treatment groups. The number of animals used in each group was kept to the minimum ($n=8$) that would still ensure adequate statistical power. This study was performed in accordance with the guidelines for animal experiments carried out in the Faculty of Agriculture and in the Graduate Course of Kyushu University, and adhered to Law no. 105 and Notification no. 6 of the government.

L- and D-Asp were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Procedure for Oral Administration Test

Following a habituation period, chicks were randomly selected and divided into four groups each consisting of 8 chicks. The birds were reared individually in experimental cages and had *ad libitum* access to diet up to the time of the experiments. On the day of the experiment, each chick (5 days old) was orally administered distilled water or L-Asp (Experiment 1) / D-Asp (Experiment 2) solutions by the elastic plastic needle on small syringe. Finkelstein *et al.* (1983) determined plasma L-Asp concentration at 0, 15, 30, 45, 60, and 120 min after applying L-Asp at 0, 1.88, 3.76, 4.89, 5.64 and 7.52 mmol/kg body weight. Plasma L-Asp concentration was hardly influenced by 1.88 and 3.76 mmol/kg. Thus, we applied here 3.75, 7.5 and 15.0 mmol/kg of L-Asp and D-Asp as the lowest, medium and highest

levels, respectively.

The birds were fed *ad libitum* diets for 2 h immediately after the treatment. Food intake (at 30, 60 and 120 min) was determined by measuring the reduction in the amount of food consumed from a pre-weighed feeder. The weight of the feeder was measured using an electric digital balance. At the end of the experiments, birds were decapitated following anesthesia with isoflurane (Mylan Inc., Japan). Blood samples were collected in heparinized tubes and centrifuged for 4 min at 4°C at 10,000 g, and the plasma was collected and stored at –80°C until analysis took place.

Analysis of Monoamines in the Brain

In Experiments 1 and 2, the brains were carefully taken from the skulls and placed on a cold glass dish. We collected the brain samples on the glass dish which was kept on dry ice. Two parts (the telencephalon and the diencephalon) were dissected from each brain, and were stored at –80°C until analysis took place. The telencephalon and diencephalon were identified according to the brain atlas of the chick (Kuenzel and Masson, 1988). Telencephalon collected is part of the brain which excludes diencephalon and mesencephalon. Diencephalon was divided by cutting between stria medullaris and posterior commissure. Diencephalon includes hypothalamus and thalamus. Monoamines (DA, 5-HT) and their metabolites, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA), were determined in these brain regions as described elsewhere (Tomonaga *et al.*, 2008), with some modifications. The tissues were weighed and homogenized in a solution of 0.2 M ice-cold perchloric acid containing 0.01 mM EDTA 2Na. Samples were allowed to sit on ice for 30 min for deproteinization. The homogenate was centrifuged at 20,000 g for 15 min. Supernatants were adjusted to pH 3 with 1 M sodium acetate and were filtered through a 0.2-μm filter (Millipore, Bedford, MA, USA). A 30-μl aliquot of filtrate was analyzed using a high-performance liquid chromatography system (Eicom, Kyoto, Japan) with a 150 × 3.0 mm ODS column (SC-50DS, Eicom) and an electrochemical detector (ECD-300, Eicom) at an applied potential of +750 mV versus an Ag/AgCl reference analytical electrode. The mobile phase was made up of an aceto-citric acid buffer (pH 3.5, 0.1 mol/l), methanol, sodium-1-octane sulfonate (0.46 mol/l) and disodium ethylenediaminetetraacetic acid (0.01 mol/l) (830:170:1.9:1). The external standard containing 100 pg/μl of each MHPG, NE, DOPAC, DA, 5HIAA, HVA and 5HT was used to identify peaks eluting in the chromatogram relating to retention time and conformation. The detection limit of the system for all monoamines was 0.1 pg/sample.

Analysis of L- and D-Asp

L- and D-Asp contents in the plasma and brains were analyzed according to the previously described method (Ohmori *et al.*, 2011). The supernatants that were collected during the monoamine analysis were applied for the measurement of L- and D-Asp contents in the brains. Plasma was prepared by centrifuging it at 20,000 × g for 15 min at 4°C (MX-307, Tommy, Japan), and then it was filtrated

through ultrafiltration tubes (Millipore, Bedford, USA). Each 20- μ l sample of the brain or plasma was dissolved with 2 μ l of 1 M NaOH and then vortexed. Both the L- and the D-Asp contents were measured by a UPLC (the Acquity™ UPLC system comprised of Waters Binary Solvent Manager, Water Sample Manager and Waters FLR Detector) with an ACCQ-TAG™ ULTRA C18 1.7 μ m 2.1 \times 100 mm column (Waters Corporation, USA). The excitation and emission wavelengths for fluorescent detection of amino acids were 350 nm and 450 nm, respectively. The system was operated with a flow rate of 0.25 ml/min at 30°C. The UPLC gradient system (A=50 mM sodium acetate (pH 5.9), B=methanol) was 10–20% B over 3.2 min, 20% B for 1 min, 20–40% B over 3.6 min, 40% B for 1.2 min, 40–60% B over 3.8 min, 60% B for 1 min, and 60–10% B over 0.01 min. Just before the analysis in UPLC, each sample (10 μ l) was transferred to a UPLC tube, and N-acetylcysteine/O-phthalaldehyde (20 μ l) and a borate buffer (70 μ l) were added; then it was left for 2 min in a dark room. The same method was used for the standard solutions. The plasma amino acid concentrations were expressed in nmol/ml, and the amino acid concentrations in the brains were expressed as pmol/mg wet tissue.

Statistical Analysis

A repeated-measures two-way ANOVA was applied for the analysis of food intake in both Experiments 1 and 2. For the other parameters, a two-way ANOVA was applied. Significant differences were denoted as $p < 0.05$. Values were presented as means \pm S.E.M. Statistical analysis was made using the commercially available package SAS (Version 9.0, SAS Institute, Cary, U.S.A., 2002). All data in each group were first subjected to a Thompson rejection test to eliminate outliers ($p < 0.01$), and the remaining data were used for the analysis among groups.

Results

Experiment 1: Effects of Oral Administration of L-Asp on Food Intake, L- and D-Asp Contents in Plasma and the Brain, and Monoamine Levels in the Brain of Chicks

Oral administration of several doses of L-Asp did not

significantly alter food intake in chicks (data not shown). Table 1 shows the effect of oral administration of several doses of L-Asp on the concentration of L- and D-Asp in plasma and the brain. A significant ($p < 0.007$) positive correlation was detected between the administered doses of L-Asp and plasma concentration of L-Asp ($-12.7[\text{SE}32.4] + 34.0[\text{SE}11.6]X$, $R^2 = 0.235$). However, administration of L-Asp had no significant effect on the plasma concentration of D-Asp. In the diencephalon, L- and D-Asp levels were not significantly changed when different doses of L-Asp were administered. In the telencephalon, significant effects on the concentration of L- and D-Asp were detected with the administration of L-Asp ($F[3, 28] = 4.04$, $p < 0.05$ for L-Asp and $F[3, 26] = 5.29$, $p < 0.01$ for D-Asp), but there was not a significant relationship between administered doses of L-Asp and L-/D-Asp levels in the telencephalon.

Changes in monoamine content in the brain after oral administration of L-Asp are shown in Table 2. DA in the diencephalon was significantly ($F[3, 27] = 3.23$, $p < 0.05$) changed, but no dose-dependent pattern was observed. No other monoamines in either the diencephalon or the telencephalon were significantly altered by oral L-Asp.

Experiment 2: Effects of Oral Administration of D-Asp on Food Intake, L- and D-Asp Contents in Plasma and the Brain, and Monoamine Levels in the Brain of Chicks

Fig. 1 shows the effects of oral administration of several doses of D-Asp on food intake. There was a significant effect of doses ($F[3, 18] = 4.36$, $p < 0.05$) and also a significant ($F[6, 36] = 2.45$, $p < 0.05$) interaction between doses and times. Food intake was reduced by oral administration of D-Asp in a dose-dependent manner, and differences among treatments were found to widen as time progressed. Significant negative correlations were detected between the administered doses of D-Asp and food intake at 30 ($p < 0.005$, $r = 0.594$), 60 ($p < 0.005$, $r = 0.572$) and 120 ($p < 0.005$, $r = 0.581$) min.

Table 3 shows changes in the contents of L- and D-Asp in plasma, the diencephalon and the telencephalon after oral administration of D-Asp. Significant increases in D-Asp

Table 1. Effects of oral administration of several doses of L-Asp on plasma and brain L- and D-Asp concentration in chicks

	L-Asp (mmol/kg body weight)			
	0	3.75	7.5	15
Plasma (nmol/ml)				
L-Asp	38 \pm 3 ^b	48 \pm 5 ^a	59 \pm 8 ^{ab}	146 \pm 47 ^a
D-Asp	2.40 \pm 0.27	2.75 \pm 0.47	2.92 \pm 0.59	3.25 \pm 0.72
Diencephalon (pmol/mg wet tissue)				
L-Asp	2,690 \pm 200	2,349 \pm 234	2,174 \pm 298	2,673 \pm 309
D-Asp	20.2 \pm 1.9	16.8 \pm 1.1	16.9 \pm 2.1	15.7 \pm 1.5
Telencephalon (pmol/mg wet tissue)				
L-Asp	3,126 \pm 200 ^b	4,273 \pm 150 ^a	3,300 \pm 367 ^{ab}	4,061 \pm 340 ^{ab}
D-Asp	32.0 \pm 2.1 ^{ab}	38.7 \pm 1.3 ^a	26.0 \pm 4.0 ^b	38.4 \pm 2.6 ^a

Means with different superscripts were significantly different at $p < 0.05$.

Values are means \pm S.E.M. The number of samples used for analysis ranged from 7 to 8.

Table 2. Effects of oral administration of several doses of L-Asp on monoamine content in the diencephalon and telencephalon in chicks

	L-Asp (mmol/kg body weight)			
	0	3.75	7.5	15
Diencephalon (pg/mg wet tissue)				
DA	181±19 ^{ab}	159±9 ^b	167±15 ^{ab}	218±9 ^a
DOPAC	109±7	94±5	100±3	108±8
HVA	101±11	86±5	85±9	93±6
5-HT	759±68	794±85	637±56	762±80
5-HIAA	440±52	386±28	390±54	359±19
Telencephalon (pg/mg wet tissue)				
DA	344±37	361±60	255±11	391±63
DOPAC	34.4±1.8	36.5±3.3	30.4±1.9	35.4±3.5
HVA	138±16	129±15	134±16	110±6
5-HT	394±23	368±28	358±14	435±26
5-HIAA	189±13	202±19	186±16	185±11

Values are means±S.E.M. The number of samples used for analysis ranged from 7 to 8. DA: dopamine, 5-HT: serotonin, and their metabolites, HVA: homovanillic acid, DOPAC: 3,4-dihydroxyphenylacetic acid, and 5-HIAA: 5-hydroxyindoleacetic acid.

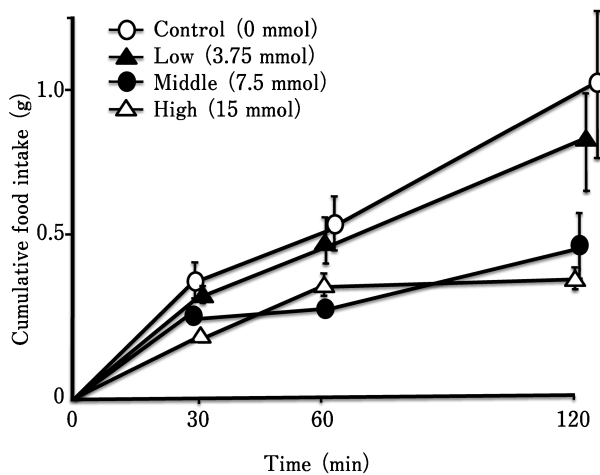


Fig. 1. The effects of several doses of D-Asp on cumulative food intake in chicks over a period of 2 h. The number of chicks used in each group ranged between 4 and 7. Values are means with S.E.M.

levels were detected in plasma ($F[3, 26]=10.22, p<0.0001$) and the diencephalon ($F[3, 23]=9.50, p<0.001$) in a dose-dependent manner, but not in the telencephalon. No significant changes in L-Asp levels were detected in plasma, or in the diencephalon or telencephalon.

As shown in Table 4, monoamine contents in both the diencephalon and the telencephalon were not altered by oral D-Asp.

Discussion

The concept that L-Asp is a neurotransmitter in the CNS is supported by a variety of data derived from studies involving rats: L-Asp is not only concentrated in nerve endings (Gundersen *et al.*, 1991), but is also localized and found

accumulated in common synaptic vesicles (Gundersen *et al.*, 1998; Fleck *et al.*, 2001). Thus, this study focused on the effects of oral L-Asp and its enantiomer D-Asp on the food intake in chicks as well as the controlling mechanism of this behavior in the brain to clarify the physiological importance of L- and D-Asp in birds.

It has been reported that there is a correlation between orally administered doses of L-Asp and consequent concentrations of L-Asp in plasma in mice (Finkelstein *et al.*, 1983; Daabees *et al.*, 1985). Thus, the effects of administered L-Asp on plasma L-Asp levels were consistent between those previously observed for mice and those observed for chicks in Experiment 1. However, food intake did not decrease significantly when chicks were orally administered L-Asp. This result was consistent with the previous reports. Bungo *et al.* (2002) reported that i.c.v. injection of L-Asp failed to affect food intake at 30, 60 and 120 min in Leghorn chicks. Other supportive data are reported by Maruyama *et al.* (1972), who demonstrated that a diet containing 6% L-Asp did not lead to significantly decreased growth of chicks. In the light of information mentioned above and of the results from the present study, it might be suggested that central or oral administration of L-Asp seemed to similar effects on food intake in chicks.

In Experiment 2, oral administration of D-Asp reduced the food consumption of chicks. Data from Experiment 2 indicate that oral administration of D-Asp caused in dose-dependent decreases food intake at short-term (30, 60 or 120 min after oral administration). The oral administration applied here seems to have a similar result with oral long-term administration of D-Asp in rat (Koyuncuoğlu *et al.*, 1982). It is clear that D-Asp has an acute effect on decreases in food intake.

It seems to be evident from the following reports that D-Asp markedly reduces food consumption. First, Koyuncuoğlu *et al.* (1982) revealed that orally administered D-Asp caused

Table 3. Effects of oral administration of several doses of D-Asp on plasma and brain L- and D-Asp concentration in chicks

	D-Asp (mmol/kg body weight)			
	0	3.75	7.5	15
Plasma (nmol/ml)				
L-Asp	35.7±8.5	42.3±12.1	25.6±8.9	7.7±5.2
D-Asp	8±2 ^b	58±26 ^b	1,170±409 ^{ab}	2,146±490 ^a
Diencephalon (pmol/mg wet tissue)				
L-Asp	1,920±90	2,221±87	2,224±175	1,876±152
D-Asp	25±6 ^c	47±8 ^{bc}	112±25 ^{ab}	166±26 ^a
Telencephalon (pmol/mg wet tissue)				
L-Asp	5,081±1,672	5,723±2,157	4,789±1,705	2,281±157
D-Asp	90±37	83±29	99±50	103±14

Means with different superscripts were significantly different at $p < 0.05$.

Values are means±S.E.M. The number of samples used for analysis ranged from 5 to 8.

Table 4. Effects of oral administration of several doses of D-Asp on monoamine content in the diencephalon and telencephalon in chicks

	D-Asp (mmol/kg body weight)			
	0	3.75	7.5	15
Diencephalon (pg/mg wet tissue)				
DA	449±33	470±68	496±61	387±37
DOPAC	44.6±3.1	51.9±5.5	53.1±2.3	47.5±2.1
HVA	73.2±4.8	72.1±11.1	77.6±2.5	79.8±7.9
5-HT	500±38	537±47	536±15	486±24
5-HIAA	224±16	241±19	273±18	263±21
Telencephalon (pg/mg wet tissue)				
DA	573±46	552±64	590±29	642±49
DOPAC	39.6±0.7	37.2±1.6	39.4±1.2	39.8±1.3
HVA	101±3	95±3	92±5	99±3
5-HT	500±9	537±11	536±13	486±17
5-HIAA	164±9	160±7	146±9	162±5

Values are means±S.E.M. The number of samples used for analysis ranged from 7 to 8.

DA: dopamine; 5-HT: serotonin; and their metabolites, HVA (homovanillic acid), DOPAC (3,4-dihydroxyphenylacetic acid), and 5-HIAA (5-hydroxyindoleacetic acid).

significant decreases in body weight and in the weight of the liver, as well as in daily food intake in rats. Second, Sugahara *et al.* (1967) concluded that D-Asp has no nutritional value and also has a retardation effect on chick growth. Third, Maruyama *et al.* (1972) found that an amino acid diet containing 2% D-Asp resulted in growth depression in chicks. Finally, Koyuncuoğlu and Berkman (1982) revealed that in rats, long-term oral administration of D-Asp caused a significant decrease in daily food intake, total body weight, and the weight of the liver and the kidneys; the concomitant oral administration of L-Asp seemed to antagonize the effect of D-Asp.

The reduced food intake following oral administration of D-Asp could be explained by four possibilities mentioned below. First, excessive D-Asp concentration may depress the food intake of chicks through unbalanced proportions of amino acids (Peng *et al.*, 1969; Harper *et al.*, 1970; Leung and Rogers, 1971). Second, the reduced food intake might

be the result of the depressed peristaltic action of the digestive tract that seemed to be caused by D-Asp administration (Maruyama *et al.*, 1972). Notably, adverse effects of D-Asp are greater in chicks fed a diet containing other D-amino acids (Kamath and Berg, 1964; Marrett and Sunde, 1965). Such effects are probably caused by a general interference in the utilization of D-amino acids (Kamath and Berg, 1964; Marrett and Sunde, 1965). Third, the concentration of D-Asp in the diencephalon increased by 6.64 times compared with the control (Table 3); although we do not know the exact mechanism by which D-Asp decreased food intake in proportion to the concentration of D-Asp in the brain, D-Asp may act as a regulator of feeding since a major feeding center, the hypothalamus, is present in the diencephalon. Here, we propose the fourth possibility. It has been hypothesized that opioid systems may be involved in the regulation of food intake concerned with acquisition of nutrients and in conservation and with expenditure of energy

(Margules, 1979). Although the endogenous opioid system stimulated feeding behavior in mammals (Bodnar, 1996) and birds (McCormack and Denbow, 1988, 1989), several other studies generally found in decreasing food intake (Kumar *et al.*, 1971; Frenk and Rogers, 1979; Marks-Kaufman and Kanarck, 1980). The decrease in food intake can be explained not only by the direct effect of opioid, but also by the opioid-induced release of antidiuretic hormone, arginine vasopressin (AVP) (George and Way, 1959; Huidobro, 1978). In fact, AVP has long been known to be capable of realizing adrenocorticotrophic hormone (ACTH) (Gillis and Lowry, 1979). Previous report revealed that the injection of D-Asp released AVP in rat (Koyuncuoğlu *et al.*, 1984).

In the light of information mentioned above, D-Asp causes a decrease in food intake probably due to the increased levels of AVP, endogenous opioid and ACTH, respectively. When both AVP and ACTH were taken into consideration together with the actions of concomitantly released beta-lipotropin and beta-endorphin, which may affect loss of appetite being opioid, the body weight loss would be a quite natural result in the rat (Koyuncuoğlu *et al.*, 1984). Arginine vasotocin (AVT), the avian homologue of mammalian AVP, is also involved in the regulation of corticosterone release mediated through the function of ACTH (Castro *et al.*, 1986). D-Asp may release AVT in the chicken as observed in AVP by the mammal. In birds, there is limited information regarding the identification of opioid receptor subtypes. It was reported that under conditions in which birds had free access to food, DAMGO, a selective μ -opioid receptor agonist, decreased food intake of chicks (Bungo *et al.*, 2004, 2005). Further experiment is necessary to clarify the mechanism.

In our previous report, i.c.v. injection of L-Asp or D-Asp clearly attenuated stress responses in chicks (Erwan *et al.*, 2012). In that study, both L- and D-Asp might directly act on the center for stress responses located in the diencephalon. On the other hand, in Experiment 1, L-Asp content in the diencephalon was not increased by oral administration of L-Asp, while in Experiment 2, D-Asp content was increased after the oral administration of D-Asp. These differences between L- and D-Asp could be explained by the specificity of the mechanism of metabolism in D-Asp: the plasma D-Asp concentration was elevated markedly (256-fold in the highest D-Asp group compared with the control group) when D-Asp was orally administered, but the increase in plasma L-Asp was limited when L-Asp was orally administered. These findings strongly support previous research described by Maruyama *et al.* (1972), who reported that free Asp concentration in plasma was elevated markedly when the D-Asp content of the diet was increased to 2 or 3%, whereas values for chicks fed L-Asp did not change so much. The accumulation of D-Asp in the plasma pool of chicks might indicate that the capacity for oxidation of this amino acid is limited. These results suggest that the metabolism of D-Asp was very slow compared with that of L-Asp. Maruyama *et al.* (1972) have proved that the removal of D-Asp from the diet returns the plasma levels of Asp to normal in chicks. In

the present study, the methods applied were limited, since the injection was done by gavage and narrow ranges of doses were applied. Further conditions should be applied to clarify the functions of L- and D-Asp in future.

When the focus was on monoamine levels in the brain, significant changes were only minimally observed in both the telencephalon and the diencephalon. In the present study, NE was not detectable, but HVA as DA metabolite and 5HT and 5HIAA were detected. These results may, at least in part, reflect release of their respective neurotransmitters (Gruss and Braun, 1997; Gruss *et al.*, 1999). Focusing on the variations in the basal values of DA content between the experiments, several studies (Saito *et al.*, 2004; Shiraishi *et al.*, 2010; Katayama *et al.*, 2010, 2011; Erwan *et al.*, 2012) have reported the fluctuation of monoamine contents especially in basal values of DA in the diencephalon or telencephalon in chicks. The reason of these differences was not clear. We conducted the same procedures when determined the monoamine contents. Due to these fluctuations among studies, we compared the data within experiments but not between experiments. However, to our knowledge there is no report of relationships between the L- or D-Asp and monoamines to explain the arousal effect of L- or D-Asp in neonatal chick. These results confirmed those of our previous study (Erwan *et al.*, 2012) in which monoamine levels in the diencephalon and telencephalon were found not to be modified by i.c.v. injection of L- or D-Asp in chicks. On the other hand, several experiments have suggested that the stimulation of NMDA receptors is linked to the regulation of the monoaminergic system in the brain (Hanania and Zahniser, 2002; Karlsson *et al.*, 2006; Hamasu *et al.*, 2009). Thus, it remains unclear whether there is a relationship between L- or D-Asp and the monoaminergic system, because monoamine levels in the brain do not always reflect the activity of their neurons. Furthermore, we only measured monoamine contents in the present study, and not monoamine release, which may be a more precise marker of monoamine neuronal activity. Further work focusing on the relationship between Asp and the monoaminergic system in the brain is necessary.

In conclusion, orally administered D-Asp, but not orally administered L-Asp, had a negative, depressive effect on food intake in neonatal chicks. D-Asp may control the feeding center without producing changes in the metabolism of monoamines.

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